

# **Early Changes in Zinc and Copper Metabolism in Rats with Alloxan Diabetes of Short Duration after Local Traumatization with Heat**

Göran Hallmans and Folke Lithner

*Departments of Pathology and Medicine, University of Umeå, Umeå, Sweden*

## ABSTRACT

Local heat trauma was induced in rats with alloxan diabetes of 3 days duration. Zinc and copper concentrations in serum, liver, heart and pancreas were estimated.

After traumatization there was a decrease of the serum zinc concentration in all animals. The lowest concentration was found in the diabetic animals. Serum copper concentrations were lower both in traumatized and nontraumatized diabetic animals compared with controls. The serum copper concentration was slightly lower in traumatized compared with nontraumatized animals in both diabetic animals and controls.

In the liver there was an increase of zinc and copper concentration in both groups of traumatized animals, but especially in the diabetic animals.

In pancreas there was a decrease in the zinc concentration in traumatized and nontraumatized diabetic animals compared with controls.

## INTRODUCTION

The effects of experimental local thermal cutaneous traumatization in non-diabetic humans and animals have earlier been studied by others (see Lithner, 1975). The skin of the lower legs in diabetics has an altered reaction compared with controls (10, 12, 13). This altered reaction is related to the occurrence of late diabetic lesions, such as microangiopathy and neuropathy, but not to the diabetic metabolic derangement per se.

To determine the importance of the metabolic derangement, we have studied by different means the early inflammatory reaction to local heat trauma in alloxan diabetic rats and rabbits (5, 11, 13, 14) without finding any obvious differences between diabetic and nondiabetic animals.

It is plausible, however, to suppose not only a late altered inflammatory reaction in diabetes (10, 11, 12, 13) but also an early one, and we have found a decrease of serum zinc concentrations after local thermal trauma to be significantly lower in diabetic compared to nondiabetic rats (6).

In the present study, we have reported in more detail about the metabolism of zinc and copper in serum, liver, pancreas and heart of alloxan diabetic rats after local traumatization with heat.

#### MATERIAL AND METHODS

Thirty male albino rats of the highly inbred R-strain were used (4). Diabetes was induced in 16 animals at the age of 3 months with an intravenous injection of alloxan, 0.34 mmol/kg, as described earlier (9). No insulin was given after the injection of alloxan. All animals had blood glucose concentrations higher than 14 mmol/l, polyuria and glucosuria. No animal had ketonuria.

Methods of depilation and anesthesia were described previously (5, 14). Traumatization was performed 3 days after the alloxan injection, the temperature used was 60°C for 5, 10 and 15 secs. Traumatization was induced in the anesthetized animals by placing the end surface of an electrically heated cylindrical brass rod, 18 mm in diameter, against the skin (10). Inspection of the skin injury was performed as described previously (5, 6).

Immediately before the alloxan injection, 4 hrs after alloxan injection, immediately before the traumatization and 8 hrs after traumatization 0.5 ml blood was obtained from the tail of each rat. After clotting, samples of serum were taken. The animals were killed 24 hrs after the traumatization and samples were then taken from serum, liver, heart (myocardium, left ventricle) and pancreas. The samples were removed with stainless steel instruments, placed in weighed, acid-washed, borosilicate tubes and the wet weights were determined. All samples, except serum, were dried at 110°C for 3 days to constant weight. The dry weights were determined and the samples were ashed for 24 hrs at 550°C. The ash was dissolved in 0.5 ml 3 mol/l HCl overnight and then diluted with 2 ml of distilled water. If necessary, the samples were further diluted with 0.6 mol/l HCl for zinc and copper determinations by atomic absorption spectrophotometry. The analyses were performed on a Varian AA-6DB spectrophotometer at 213.9 nm and 324.7 nm for zinc and copper, respectively. Reference samples of the minerals in 0.6 mol/l HCl were used. Samples of serum were diluted 10 times in 0.1 M HCl before atomic absorption spectrophotometry with reference samples in 0.1 M HCl.

Differences between groups were tested using Student's t-test for unpaired observations. The test was modified if the variances were significantly different ( $p < 0.01$ ; F-test). Differences between groups were also tested with the nonparametric rank sum test of Wilcoxon.  $P < 0.05$  was chosen as the level for statistical significance.

## RESULTS

### Zinc and copper concentrations

Alloxan injection did not significantly affect the serum zinc concentrations (Table 1).

Table 1. Zinc concentration in serum before and after injection of alloxan or sodium chloride. The results are given as the mean  $\pm$  SE. No significant differences were observed between the four groups.

	Concentrations $\mu\text{g Zn/g serum}$	Number n
Before NaCl injection	$1.33 \pm 0.06$	14
4 hrs after NaCl injection	$1.31 \pm 0.06$	14
Before alloxan injection	$1.41 \pm 0.05$	16
4 hrs after alloxan injection	$1.25 \pm 0.09$	16

Eight hrs after traumatization a decrease in serum zinc concentration was found both in controls ( $p < 0.05$ ) and in diabetic animals ( $p < 0.001$ ). The decrease was most pronounced in the diabetic animals (Fig. 1) and 24 hrs after traumatization the decrease persisted in them.

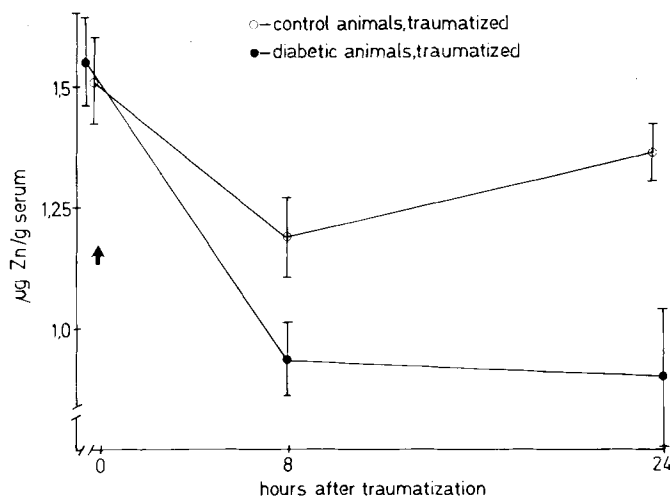


Fig. 1. The effect of cutaneous traumatization (arrow) by heat on serum zinc concentration. The results are presented as the mean  $\pm$  SE. T-test results, control animals - diabetic animals: 8 hrs  $p = 0.06$ . 24 hrs  $p < 0.02$ .

The copper concentrations in serum were only registered 24 hrs after traumatization and they were significantly lower both in traumatized and nontraumatized diabetic rats compared with controls (Fig. 2). Lower concentrations of serum copper were seen in traumatized diabetic and nondiabetic animals compared with nontraumatized animals, but only significantly lower in controls.

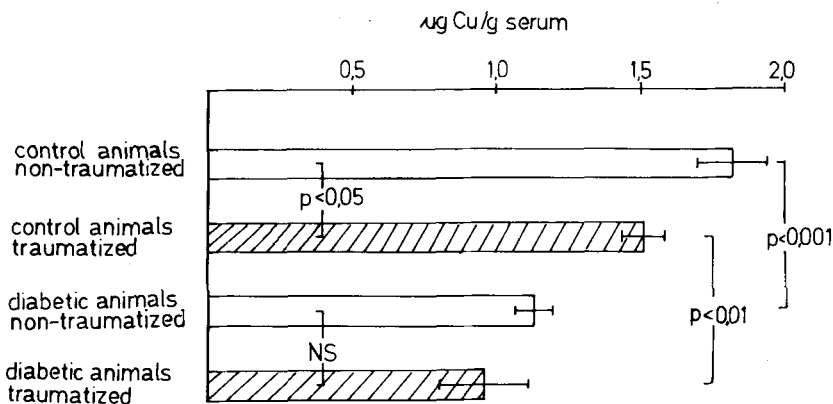


Fig. 2. The effect of alloxan injection and cutaneous traumatization by heat on serum copper concentration. The results are presented as the mean  $\pm$  SE. T-test results are indicated in the figure.

An increase of the zinc concentrations in the liver was observed in diabetic animals compared with controls and in traumatized animals compared with non-traumatized (Fig. 3). The changes of the copper concentrations in the liver were similar to those of zinc, however, they were not significant except in traumatized diabetic animals compared with controls (Fig. 4).

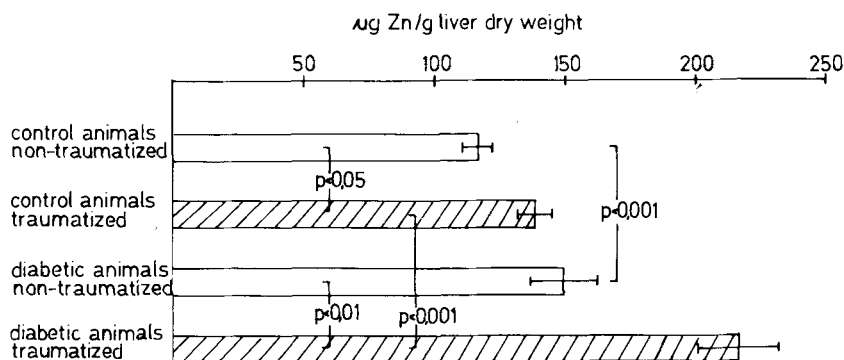


Fig. 3. The effect of cutaneous traumatization by heat on the zinc concentration in the liver. The results are presented as the mean  $\pm$  SE. T-test results are indicated in the figure.

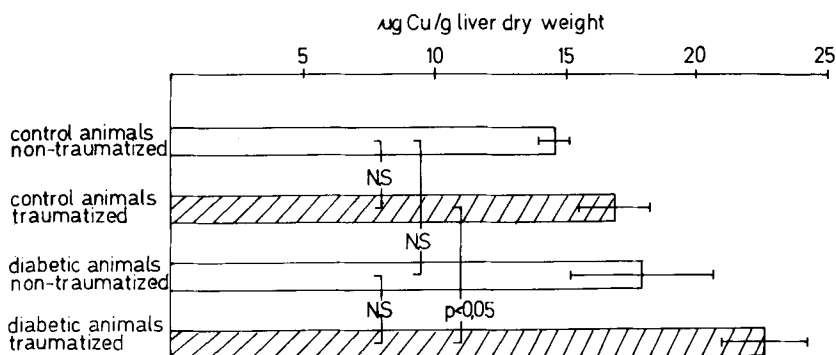


Fig. 4. The effect of cutaneous traumatization by heat on the copper concentration in liver. The results are presented as the mean  $\pm$  SE.

In diabetic animals after traumatization there was a decrease in the pancreatic zinc concentrations compared with controls (Table 2). Only a slight decrease was seen in the copper concentrations of diabetic animals compared with controls.

In the heart no differences were seen, neither in zinc nor in copper concentrations between groups (Table 2).

Table 2. Zinc and copper concentrations in pancreas and heart ( $\mu\text{g/g}$  dry weight). The results are given as the mean  $\pm$  SE. The results of comparisons with non-traumatized control animals (t-test) are indicated.

	$\mu\text{g Zn/g dry weight}$			
	control animals		diabetic animals	
	non-traumatized	traumatized	non-traumatized	traumatized
Pancreas	110.8 $\pm$ 4.4	101.4 $\pm$ 5.6	81.0 $\pm$ 3.5***	85.3 $\pm$ 7.7*
Heart	77.8 $\pm$ 1.2	72.3 $\pm$ 0.8	71.0 $\pm$ 1.7	70.2 $\pm$ 1.2
	$\mu\text{g Cu/g dry weight}$			
	control animals		diabetic animals	
	non-traumatized	traumatized	non-traumatized	traumatized
Pancreas	7.4 $\pm$ 0.7	5.3 $\pm$ 0.3*	6.6 $\pm$ 0.4	6.1 $\pm$ 0.5
Heart	21.8 $\pm$ 0.6	20.1 $\pm$ 0.9	20.3 $\pm$ 1.7	19.4 $\pm$ 1.0

\*  $p < 0.05$

\*\*\*  $p < 0.001$

### Macroscopic and microscopic findings in traumatized areas

A slightly higher, but not statistically significant, extent of purpura was seen in controls compared with diabetic animals 8 and 24 hrs after the traumatization. Similar findings were described previously (6).

Microscopically, no differences were found between controls and diabetic animals.

There was extensive tissue damage to the skin after 10 and 15 secs of traumatization, often with necrosis through the entire thickness of the skin including the panniculus carnosus. There was an accumulation of granulocytes and mononuclear cells surrounding this devitalized area. After 5 secs of traumatization the epithelium showed signs of disruption and necrosis with accumulation of inflammatory cells, mostly granulocytes, in the upper dermis. Signs of inflammation were sometimes seen to extend to the panniculus carnosus.

### DISCUSSION

The serum zinc concentration decreased after heat trauma both in diabetic animals and in controls while the liver concentration of zinc increased. This relationship has been postulated to be due to the liberation of a leucocytic endogenous mediator (LEM) from activated phagocytic cells (6) which, in the present study, were located in the traumatized areas. Several functional disturbances in diabetic granulocytes have been reported (see Bagdade et al. 1978).

The decrease of serum zinc concentrations was more pronounced in the diabetic animals, and this has been reported previously (6). This can explain the more pronounced increase of zinc concentrations in the liver of traumatized diabetic animals. However, an increase of the zinc concentrations in the liver was also seen in nontraumatized diabetic animals when compared with controls. This indicates that an increased concentration of zinc binding sites is found in the liver of the diabetic animal. This may be due to an increased synthesis or an incorporation of a zinc binding ligand in diabetic animals in response to the metabolic derangement or to the injection of alloxan.

The increase of zinc concentrations in the liver in traumatized controls and in nontraumatized diabetic animals suggests that these animals need more zinc than what is available from the loosely bound serum zinc. Partly, it comes from an increased absorption from the intestine (7) and partly probably from zinc mobilized from tissues of other organ systems. In this study, however, no significant decreases were observed in the zinc concentrations of pancreas or heart after traumatization, but the zinc concentrations of nontraumatized diabetic animals were decreased compared with the corresponding controls.

Most of the serum copper is strongly bound to ceruloplasmin, an  $\alpha$ -2-globulin with oxidase activity (3, 8). A minor part of the serum copper is weakly

bound to albumin and amino acids (3). Ceruloplasmin belongs to the group of acute phase proteins. Elevation of ceruloplasmin and serum copper is usually induced by acute stress (1) but we should'nt expect it to be present as soon as 24 hrs after the thermal trauma, as in our study. The decrease of the serum copper concentration in the diabetic animals probably is due to a decrease of ceruloplasmin concentration. The liver plays an important role in copper homeostasis and 3 of it's functions in this respect are: storage of copper, ceruloplasmin synthesis and excretion of copper through the bile (3). At least one of these functions has to be altered after traumatization in the diabetic animals which had increased copper concentrations in their livers.

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Address for reprints:

Folke Lithner, M.D.

Department of Medicine

University of Umeå

S-901 85 Umeå

Sweden